Effectiveness of Brown Algae (*Padina australis*) Extract as Antioxidant Agent

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Effectiveness of Brown Algae (*Padina australis*) Extract as Antioxidant Agent

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Abstract. The pattern of disease development has shifted from infectious diseases to degenerative diseases that are triggered by a free radical. The body needs an antioxidant that able to ward off free radicals. One of the potential natural resources is brown algae that contain bioactive compounds and widely used in medicine and the pharmaceutical industry. This research aims to identify the effectiveness of brown algae (*Padina australis*) extract as a natural antioxidant agent. This research was carried out in several steps, including algae extraction by sonicator method, phytochemical test to identify secondary metabolites in extract and antioxidant test by using DPPH method. The results showed that the extract of brown algae (*Padina australis*) contained flavonoids, alkaloids, saponin, and steroids. It also has antioxidant activity with an IC_{50} value of 102,590 μg/mL.

1. Introduction

Changes in lifestyle and dietary also cause patterns of disease development to shift toward degenerative diseases such as tissue inflammation, cancer, and cataracts that are triggered by free radicals that damage cell components such as DNA, lipids, proteins, and carbohydrates. The damage can cause various biological disorders such as atherosclerosis, cancer, diabetes, and other degenerative diseases. Although the body has its immune system, if the amount of free radicals is excessive, it needs an additional exogenic substance or compound known as an antioxidant such as flavonoid compounds, vitamin A, vitamin C, and vitamin E [1].

Sources of antioxidant can be explored from natural materials, especially those sourced from the sea considering that Indonesia is an archipelagic country that has a very long coastline reaching approximately 99,093 km. Seaweed or algae is one of the abundant biological resources in the Indonesian ocean, where there are 555 species of seaweed that have been found [2]. Research on the secondary metabolite content of seaweed, both extract, and pure compounds, shows a variety of antibacterial, antiviral, antiparasitic, antifungal, cytotoxic and anticancer activities [3]. An example of natural material that widely used is brown algae (*Padina australis*).

Brown algae are one of the potential types of algae because they contain alkaloids, glycoside, tannin, steroid, phenolic and flavonoids that have been known to be widely used in medicine and the pharmaceutical industry. Also, phenolic compounds and flavonoids that are also found in brown

seaweed has antioxidant activity [4]. Brown algae also contain fucoxanthin which has efficacy as anticancer, antioxidant, antiobesity and antidiabetic [5]. This makes algae one of the most potent natural resources to be developed. Therefore, in this research, algae extraction will be carried out to later being studied how its effectiveness as an antioxidant.

2. Experimental

2.1. Material

The materials of this research were brown algae (*Padina australis*) from Takalar, South Sulawesi, magnesium (Mg) powder, sulfuric acid (H_2SO_4), chloroform ammonia, $FeCl_3$ 1%Wagner reagent, chloroform ($CHCl_3$), DPPH, distilled water, Whatman 42 filter paper.

2.2. Method

2.2.1. Extraction.

Fresh brown algae (*Padina australis*) were washed and then cut into pieces. After that, the algae were air-dried and ground using a grinder. The pollen algae then extracted with methanol solution 1:10 (b/v). The extraction process was by using a sonicator with temperature 45 ^oC for 20 minutes. Extract from the extraction process was filtered and evaporated to obtain the thick extract.

2.2.2. Phytochemical test.

The extract that obtained was continued to the phytochemical test. Phytochemical screening for alkaloid, phenolic, flavonoid, saponin and terpenoid/steroid was performed according to the standard procedure described by Harbone, et al. (1987).

2.2.3. Antioxidant activity test.

The antioxidant activity was identified by using DPPH method. Extract algae was made in various concentration 10, 20, 40, 80 and 160 ppm. DPPH 0.4 mM was then 1mL added into those various extract solution and left in a dark space to incubate for 30 minutes. The inhibition ability of extract towards DPPH was measured using UV-Vis at wavelength 515 nm.

3. Result and Discussion

3.1. Extraction and phytochemical screening

The extraction process began with cleaned up the algae then ground to reduce the sample size while increasing the surface area, so that interaction with the solvent in the extraction process can be maximized. The extraction process was by using a sonicator. This method was chosen because it has several advantages, such as fast process because it uses ultrasonic waves, easy, safe, the amount of solvent and the sample used is small so that it can minimize the waste and furthermore can increase the amount of rough yield from the extracted sample. The extraction process also did not use high temperature because bioactive components such as flavonoid, tannin and phenol are damaged at a temperature above 50^oC [6]. The extract obtained was then evaporated using an evaporator to obtain a thick extract. The thick extract was continued to phytochemical test.

Phytochemical test was carried out to identify the content of secondary metabolites in the extract. Phytochemical test includes terpenoid/steroid, alkaloid, phenolic, flavonoid, and saponin. The results of the phytochemical test on brown algae (*Padina australis*) extract can be seen in Table 1. The results obtained are in accordance with some previous researchers such as Maharany, et al (2017) and Haryani, et al (2014) who also identified the presence of secondary metabolite compounds such as flavonoid, steroid, alkaloid, and saponin in brown algae (*Padina australis*).

Positive result for alkaloid by Wagner test is characterized by the formation of light brown to red precipitate thought as potassium-alkaloid. In making Wagner's reagent, iodine reacts with ion I from potassium iodide to produce a brown I^3 ion. In the Wagner test, K^+ metal ions will form covalent

coordinate bonds with nitrogen in the alkaloids to form a precipitating potassium-alkaloid complex. The identification of flavonoids using the Wilstater test showed a change in color to orange which means positive the presence of flavonoids. Magnesium and hydrochloric acid in the Wilstater test react to form bubbles which are H^2 gas while concentrated HCl and Mg metals in this test are function to reduce the benzopiron nucleus contained in the flavonoid structure, so that the color changes to red or orange[7].

Table 1. Result of phytochemical test

Secondary metabolite	Test result
Alkaloid	+
Terpenoid / Steroid	+
Phenolic	-
Flavonoid	+
Saponin	+

Steroid and triterpenoid tests were carried out using the Liebermann-Bouchard method. The triterpenoid reaction with the Liebermann-Bouchard reagent will produce a red-purple color, while the steroid will give a green-blue color. This is based on the ability of triterpenoid compounds and steroids to form color by H_2SO_4 in the acetic acid anhydride solvent. In this experiment, a bluish-green discoloration occurred in the extract tested, so that the extract was positive contained steroid. The extract is positive contains phenol if there is a blackish blue color change after 1% iron (III) chloride ($FeCl_3$) addition. Phenolic will react with 1% $FeCl_3$ to form a solid red, purple, blue, or black color because $FeCl_3$ reacts with the aromatic -OH group. The colored complex formed is thought to be iron (III) hexafenolate[8].

In the saponin test, a stable foam was formed on the extracted sample after the addition of distilled water and shaken. Hydrolysis reaction is the principle of the saponin test, which in water saponin compounds will form foam due to the hydrolysis process. Extract is positive contain of saponin if the foam formed remains stable ± 7 minutes [9].

3.2. Antioxidant activity

The antioxidant activity of the brown algae (*Padina australis*) extract was tested using the DPPH method (Fig.1). DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. When DPPH is mixed with a substrate acting as a hydrogen atom donor, a stable non-radical form of DPPH is obtained, with the simultaneous change in the color of the solution from violet to pale yellow [10]. The antioxidant ability was measured by calculating the IC_{50} value.

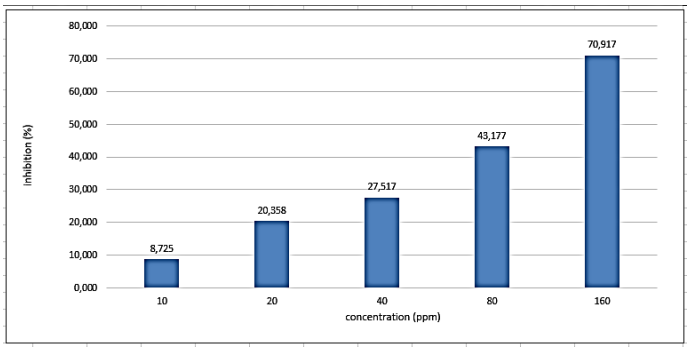

Figure 1. Inhibition ability of brown algae extract

The test result showed that the brown algae extract can inhibit the free radical DPPH for 70,917% and had a moderate category of antioxidant activity with an IC_{50} value of 102.590 µg/mL. Brown algae extract has the potentiality as a natural antioxidant agent because it contains bioactive compounds such as flavonoid, alkaloid, saponin, and pigment such as fucoxanthin [11]. Free radicals are unstable atoms, so to be stable radical compounds will look for donor molecules. If the donor molecule has given its electron, then the donor molecule will become an unstable molecule, then this molecule will look for electrons from other molecules and so on. The principle of antioxidants is to donate hydrogen to radical compounds so that non-radical compounds will be formed (Fig.2) [12].

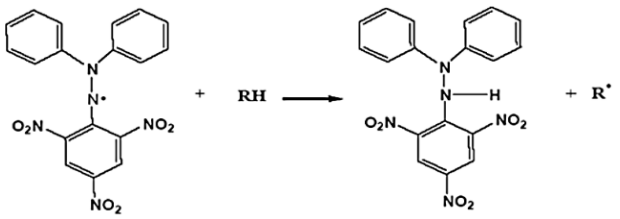

Figure 2. Common reactions of DPPH inhibition by antioxidant

This result was related to Hidayati, et al (2017) that also obtained the antioxidant activity with IC_{50} value of 137.02 µg/mL and Maharany, et al (2017) with IC_{50} value of 87,082 µg/mL.

4. Conclusion

The secondary metabolite compounds contained in brown algae (*Padina australis*) extract sourced from Takalar, South Sulawesi were alkaloid, steroid, flavonoid and, saponin. Brown algae (*Padina australis*) also potential as a natural antioxidant agent with IC_{50} value of 102.590 µg/mL.

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